STUDIES ON THE ANAEROBIC METABOLISM AND THE AEROBIC CARBOHYDRATE CONSUMPTION OF SOME FRESH WATER SNAILS

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Field work attempting the eradication of schistosome-transmitting snails in various parts of the world has demonstrated the inadequacy of the hitherto available molluscacides. If control of schistosomiasis is to be attempted by means of chemical compounds interfering with the metabolic pathways of the intermediate hosts of the flukes, the establishment of a theoretical basis for further evaluation of potential and actual molluscacides is urgently needed.

In a previous paper by von Brand, Nolan, and Mann (1948), some data on the aerobic faculties of various species of snails are recorded. A detailed knowledge of the anaerobic metabolism of such snails is also necessary since, according to a personal communication by Dr. W. H. Wright, some schistosome-transmitting snails have the ability to escape the action of poisons by burrowing into the mud, which is usually very poor in oxygen.

In the present paper an attempt is made to answer some unsolved problems concerning the anaerobic metabolism of snails which, in fact, has so far never been studied. Specifically, the following points have been investigated: Anaerobic resistance of various species, the anaerobic carbohydrate consumption, carbon dioxide and lactic acid production, and the quantitative relationships between anaerobic and aerobic carbohydrate consumption.

Material and Methods

The following species of snails were employed and where no further information is provided they were of the same derivation as those used in a previous study (von Brand, Nolan, and Mann, 1948).

1. Pulmonates

Planorbidae: *Australorbis glabratus; Helisoma duryi; Helisoma trivolvis* specimens collected near Brownsville, Texas; *Tropicorbis obstructus; Tropicorbis donbilli; Planorbarius corneus,* laboratory-reared; *Biomphalaria boissyi,* laboratory-reared from Egyptian specimens; *Biomphalaria pfeifferi,* laboratory-reared from Liberian stock.

Lymnaeidae: *Lymnaea stagnalis* and *Lymnaea palustris,* both laboratory-reared from Douglas Lake, Michigan stock; *Lymnaea natalensis,* laboratory-reared from Dharan, Saudi Arabia, specimens.

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PHYSIDAE: *Physa gyrina; Physa cubensis,* laboratory-reared from Cuban stock; *Aplexa nitens,* laboratory-reared from specimens collected near Brownsville, Texas.

2. Operculates

AMNICOLIDAE: *Oncomelania nosophora,* laboratory-reared from Japanese stock.

POMATIOPSIDAE: *Pomatiopsis lapidaria.*

THIARIDAE: *Melanoides tuberculatus,* laboratory-reared from Dharan, Saudi Arabia, specimens.

PLEUROCERIDAE: *Goniobasis livescens,* used shortly after being shipped from northern Michigan.

All determinations were carried out at 30° C., a temperature corresponding approximately to the summer temperature in the Washington, D. C., area. It was well tolerated by all snails.

Warburg equipment was used throughout the work, flasks of about 17 and 5 ml. being employed for larger and smaller snails respectively. Two ml. of dechlorinated tapwater served as medium in the former, one ml. in the latter. Anaerobic conditions were established by passing a stream of 99.99 per cent pure Linde nitrogen, further purified by passing over heated copper, through the shaking flasks for 15 to 20 minutes.

In the aerobic experiments the previously described technique was again employed (von Brand, Nolan, and Mann, 1948).

The total carbohydrates were determined in the snail tissues by Dische and Popper's (1926) method, the color being read in a Fisher electrophotometer with a filter at 425 mμ, instead of in a visual colorimeter.

Barker and Summerson's (1941) method was used for the lactic acid determinations in the snail tissues and the medium.

RESULTS

1. *Anaerobic resistance*

Considerable differences in anaerobic resistance between various groups of snails occurred. The figures summarized in Table I show that the Lymnaeidae and Physidae withstood anaerobic conditions without apparent damage for only 6 hours, and that most died within 16 hours. The Planorbidae and the operculate snails were more resistant, most species surviving regularly for at least 24 hours, and some, especially *Helisoma trivolvis* and *Melanoides tuberculatus,* for 48 to 64 hours. *Australorbis glabratus* and *Biomphalaria pfeifferi,* however, were somewhat more sensitive, all specimens surviving regularly only 16 hours in the lack of oxygen.

The behavior of the snails under anaerobic conditions was quite characteristic. All extended maximally out of their shells and soon, at the latest within a few hours, became completely motionless. If not used for chemical determinations, the snails, after the end of the anoxic period, were placed into beakers containing aerated dechlorinated tapwater. As long as they were fully extended, they recovered completely, resuming motion soon after restoration of aerobic conditions. If the anaerobic period lasted too long, on the contrary, the snails began to hemorrhage and finally retracted into their shells. This seemingly indicates that the above-

mentioned lack of motion was not a complete paralysis. Snails which had retracted into their shells during the anaerobic period did not recover during a subsequent aerobic period. Whether, in all cases, they actually died during the anaerobic period, or died shortly thereafter, could not be determined.

2. *Anaerobic metabolism*

A condensed summary of our experimental data (Table II) shows that, in general, two series were carried out with a given species of snails. In one series the CO_2 production and carbohydrate consumption were studied. In the second series the CO_2 production, the lactic acid content of the tissues, and the excretion of lactic acid into the medium were determined. The carbon dioxide determination then was common to both series. A survey of the figures shows that in pulmonates the

TABLE I

Anaerobic resistance of various species of fresh water snails at 30° C.

Species	Period of anaerobiosis									
	6 hours		16 hours		24 hours		48 hours		64 hours	
	Number of specimens	Per cent surviving	Number of specimens	Per cent surviving	Number of specimens	Per cent surviving	Number of specimens	Per cent surviving	Number of specimens	Per cent surviving
Australorbis glabratus	72	100	59	98	12	25	12	0		
Helisoma duryi					36	100				
Helisoma trivolvis			6	100	6	100	6	100	5	100
Tropicorbis obstructus					110	100			12	25
Tropicorbis donbilli	12	100			12	66	6	0		
Planorbarius corneus	36	100			42	100			12	17
Biomphalaria boissyi	12	100	36	100	12	100	12	0		
Biomphalaria pfeifferi			67	100	12	50	12	0		
Lymnaea stagnalis	36	100	12	9						
Lymnaea palustris	36	100	12	0						
Lymnaea natalensis	48	100	12	0						
Physa gyrina	64	100	33	3						
Physa cubensis	72	100	6	0						
Aplexa nitens	36	97	12	0						
Oncomelania nosophora					30	100	18	17		
Pomatiopsis lapidaria					108	98	12	25		
Melanoides tuberculatus					60	100	12	92	12	84
Goniobasis livescens					12	100				

CO_2 figures of both series were in most cases in reasonably close agreement, while considerably greater differences occurred in this respect in the two operculates *Pomatiopsis lapidaria* and *Melanoides tuberculatus*, so tested. No definite reason for the irregular behavior of these latter can be given.

The length of the anaerobic period was chosen for each species in accordance with the resistance data discussed in the preceding section. In the cases of *Australorbis glabratus* and *Planorbarius corneus*, two periods of different duration were tested in order to see if significant differences in metabolism occurred. The shorter period corresponded to that used with the nonresistant species. The data (Tables II and III) gained from both periods agreed fairly well, with the exception of an unexplained larger excretion of lactic acid by Planorbarius during the shorter period. There is, therefore, no reason to assume that the differences in metabolism between

resistant and nonresistant species mentioned below are correlated with the different lengths of anaerobic periods employed.

From the data summarized in Table II, the average hourly rate of metabolic processes has been calculated (Table III), and a perusal of both Tables II and III reveals the following facts.

Total carbohydrates. The highest carbohydrate content, 3.5 per cent of the fresh weight, was found in *Lymnaea natalensis,* while *Physa gyrina* showed the lowest, 1 per cent. The rates of anaerobic carbohydrate consumption of well-fed snails were highest in the Physidae and Lymnaeidae, intermediate in most Planorbidae, and lowest in operculates. The nutritional state of the snails was of consid-

TABLE II

Anaerobic metabolism of various species of snails

Species	No. of exper.[1]	Anaerobiosis	Entire exper. period. Carbon dioxide mm.[3] per 1 gm.[1]	Carbohydrates in per cent		Lactic acid in tissues microgram per gm.		Lactic acid excreted microgram per gm.
		Hours		Initial	Final	Initial	Final	
Australorbis glabratus[2]	24; 24	16	641 ±20; 664 ±26	2.69 ±0.18	2.19 ±0.15	232 ±21	155 ± 29	422 ±121
Australorbis glabratus[2]	36; 36	6	187 ± 9; 203 ±14	2.58 ±0.19	2.47 ±0.15	150 ±22	150 ± 18	118 ± 25
Australorbis glabratus[3]	18	16	327 ±22	1.35 ±0.06	1.11 ±0.12			
Australorbis glabratus[4]	22	16	219 ±27	0.54 ±0.12	0.32 ±0.05			
Helisoma duryi	18; 18	24	819 ±36; 528 ±35	2.08 ±0.18	1.00 ±0.13	106 ±15	104 ± 19	114 ± 36
Tropicorbis obstructus	17; 18	24	1177 ±43; 1024 ±56	1.77 ±0.13	0.67 ±0.07	160 ±34	148 ± 21	357 ± 37
Planorbarius corneus	24; 17	24	760 ±44; 709 ±33	2.94 ±0.02	2.65 ±0.18	267 ±66	202 ± 38	131 ± 32
Planorbarius corneus	18; 24	6	141 ±12; 143 ±12	2.35 ±0.34	2.27 ±0.29	285 ±12	205 ± 11	252 ± 14
Biomphalaria boissyi	18; 17	16	646 ±35; 637 ±23	2.59 ±0.17	1.70 ±0.13	320 ±20	265 ± 24	692 ± 60
Biomphalaria pfeifferi	18; 18	16	752 ±45; 637 ±37	2.92 ±0.19	2.07 ±0.23	229 ±23	207 ± 32	506 ± 86
Lymnaea stagnalis	18; 18	6	479 ±34; 567 ±61	1.26 ±0.10	0.99 ±0.11	247 ±47	689 ±102	871 ±109
Lymnaea palustris	18; 18	6	300 ±34; 395 ±46	1.22 ±0.13	0.71 ±0.07	112 ±16	664 ± 87	34 ± 5
Lymnaea natalensis	18; 18	6	284 ±20; 237 ±24	3.55 ±0.17	3.25 ±0.10	302 ±48	756 ± 97	1000 ± 77
Physa gyrina	18; 17	6	144 ±11; 214 ±23	0.98 ±0.07	0.49 ±0.06	85 ± 9	313 ± 41	45 ± 9
Physa cubensis	17; 18	6	313 ±35; 395 ±46	1.22 ±0.11	0.76 ±0.08	185 ±34	590 ±161	63 ± 8
Aplexa nitens	17; 18	6	277 ±19; 371 ±21	2.05 ±0.10	0.94 ±0.11	185 ±17	384 ± 35	679 ± 66
Oncomelania nosophora	18	24	316 ±35	1.42 ±0.12	0.99 ±0.08			
Pomatiopsis lapidaria	12; 16	24	290 ±42; 646 ±89	1.47 ±0.08	1.05 ±0.08	114 ±25	98 ± 25	188 ± 45
Melanoides tuberculatus	18; 29	24	322 ±35; 177 ±24	2.73 ±0.13	2.37 ±0.13	89 ±20	128 ± 24	120 ± 34
Goniobasis livescens	12	24				97 ±13	93 ± 7	191 ± 24

[1] The first figures in these columns refer to the carbohydrate series; the second figures to the lactic acid series.

[2] Well-fed snails.

[3] Snails from an aquarium with scanty food supply.

[4] Snails starved for 2 weeks prior to the determinations.

The figures behind the plus and minus signs represent the standard error of the mean.

erable influence as is seen by the example of *Australorbis glabratus* which, after 2 weeks' starvation, consumed less than half the amount of endogenous carbohydrate catabolized by well-fed specimens.

Lactic acid. The lactic acid content of the tissues of snails taken from aerated aquaria was relatively low, varying from about 9 mg. per cent in *Physa gyrina* or *Melanoides tuberculatus* to about 30 mg. per cent in *Lymnaea natalensis.* In contrast to these relatively small variations in preanaerobic lactic acid level, a very distinct difference in the anaerobic lactic acid levels was observed in different species. The Lymnaeidae and Physidae showed a marked accumulation of lactic acid within their tissues, while this did not occur in the Planorbidae and the operculate snails. In most species belonging to these latter groups the anaerobic level was even lower

than the aerobic one. While most of these latter differences do not appear statistically significant, their repeated occurrence suggests a lactic acid excretion during anaerobiosis in excess of the amounts formed.

All species studied excreted lactic acid. The total production of lactic acid during the anaerobic period (lactic acid excreted plus lactic acid accumulated in the tissues, or lactic acid excreted minus lactic acid deficit in the tissues) was largest in Lymnaeidae and Physidae, but small in the other species.

TABLE III

Hourly anaerobic metabolic changes of various species of snails per 1 gm. tissue

Species	CO_2 mm.[3] produced	Carbohydrate consumed, mg.	Lactic acid accumulated in tissues, mg.	Total lactic acid produced, mg.	Carbon balance per cent of total CO_2 liberated from bicarbonate by lactic acid	Per cent of consumed carbohydrate carbon accounted for by lactic acid
Australorbis glabratus [1]	41	0.31	0	0.022	13	7
Australorbis glabratus [2]	33	0.18	0	0.020	15	11
Australorbis glabratus [3]	20	0.15				
Australorbis glabratus [4]	14	0.14				
Helisoma duryi	28	0.45	0	0.005	4	1
Tropicorbis obstructus	46	0.46	0	0.014	8	3
Planorbarius corneus [5]	30	0.12	0	0.003	3	3
Planorbarius corneus [6]	24	0.13	0	0.029	29	22
Biomphalaria boissyi	40	0.56	0	0.040	25	7
Biomphalaria pfeifferi	43	0.53	0	0.030	17	6
Lymnaea stagnalis	87	0.45	0.074	0.219	63	49
Lymnaea palustris	58	0.85	0.092	0.098	41	12
Lymnaea natalensis	43	0.50	0.076	0.243	139	50
Physa gyrina	30	0.82	0.038	0.046	38	5
Physa cubensis	59	0.77	0.068	0.078	32	10
Aplexa nitens	54	1.85	0.033	0.146	67	8
Oncomelania nosophora	13	0.18				
Pomatiopsis lapidaria	20	0.18	0	0.007	9	4
Melanoides tuberculatus	10	0.15	0.002	0.007	17	5
Goniobasis livescens	9		0	0.008		

[1] Well-fed snails, 16 hours anaerobiosis.
[2] Well-fed snails, 6 hours anaerobiosis.
[3] Snails from an aquarium with scanty food supply.
[4] Snails starved for 2 weeks prior to the determinations.
[5] Well-fed snails, 24 hours anaerobiosis.
[6] Well-fed snails, 6 hours anaerobiosis.

The last column of Table III shows conclusively that lactic acid is, from a quantitative standpoint, a major end product of the anaerobic carbohydrate metabolism only in *Lymnaea stagnalis* and *Lymnaea natalensis*. In all other species, unidentified end products must predominate by far.

Carbon dioxide. The carbon dioxide excretion showed fairly large variations from species to species. Due to the calcareous shells it was, unfortunately, impossible to study the question of carbon dioxide retention in the tissues. Although the CO_2 figures are probably not absolutely correct, it is believed that the following conclusions are not far from the truth.

The next to the last column of Table III shows that in Lymnaeidae and Physidae a major portion of the carbon dioxide excreted was probably of inorganic origin, having been liberated from bicarbonate during glycolysis leading to lactic acid. In *Lymnaea natalensis* even more lactic acid was found than corresponded to the carbon dioxide excreted. In all other species the amount of CO_2 due to lactic acid was only moderate. In view of the absence of information concerning the other end products of the anaerobic carbohydrate metabolism, the question of whether or not the remaining CO_2 was also of inorganic origin or whether it represented true respiratory CO_2 must remain unanswered at the present time.

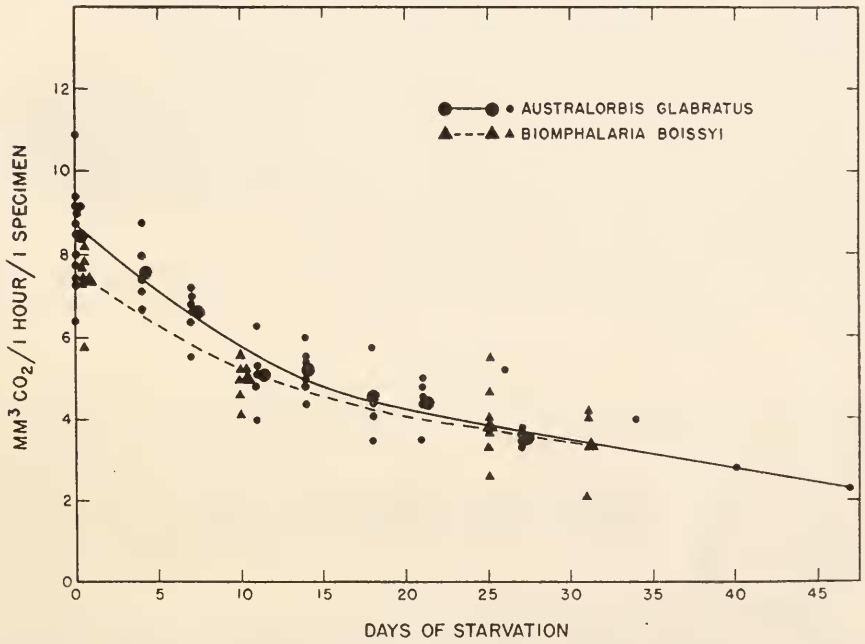

FIGURE 1. Influence of starvation on the anaerobic carbon dioxide production of aquatic pulmonate snails. Twelve well-fed specimens of *Australorbis glabratus* and 6 specimens of *Biomphalaria boissyi* were used. They were exposed on specified days to 6 hours anaerobiosis. Between the anaerobiosis periods the snails were kept starving in aerated, dechlorinated tap-water. The determinations were continued until the last snail of each group died. The figure shows the individual and the mean values (small and large symbols respectively).

During starvation (Fig. 1) the rate of anaerobic CO_2 production fell progressively but the decline was, on the whole, less pronounced than in the case of the previously studied aerobic metabolism (von Brand, Nolan, and Mann, 1948).

In several species the specimens used for the CO_2 determinations varied rather markedly in size. A study of this material (Fig. 2) shows that in intraspecific comparisons the CO_2 production followed the surface law rather well (relative surface calculated as weight $^2/_3$), while the same obviously did not hold true for all cases of interspecific comparison.

3. *Aerobic carbohydrate metabolism*

The data summarized in Table IV demonstrate that all species consumed carbohydrate under aerobic conditions. The aerobic rate was always smaller than the anaerobic one as indicated by the ratio between anaerobic and aerobic rate given in the last column of Table IV. It is noteworthy, however, that the quotient was small in 5 out of the 7 species tested.

In *Planorbarius corneus* and *Pomatiopsis lapidaria* the amount of oxygen consumed by the snails was clearly in excess of the amount required for complete oxidation of the consumed carbohydrate. In the other cases it was either just

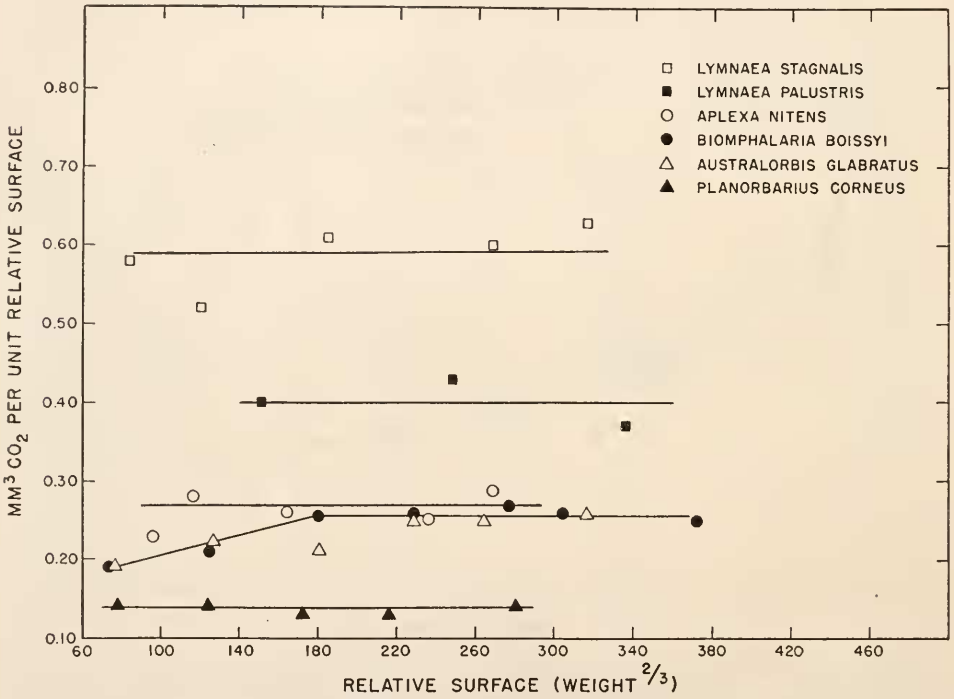

FIGURE 2. Relation between anaerobic carbon dioxide production and relative surface of pulmonate aquatic snails. The values are mean values of groups of snails varying in number between 3 and 20.

sufficient to account theoretically for complete oxidation, or insufficient. It must be assumed that in these latter cases some incomplete oxidations occurred. This is probably also true for *Australorbis glabratus* and *Helisoma duryi* since it is unlikely that they metabolize carbohydrate exclusively. The previously reported data concerning the respiratory quotient of *Australorbis glabratus* (von Brand, Nolan, and Mann, 1948) exclude an assumption of exclusive carbohydrate utilization, at least for this species.

The lactic acid level of the snail tissues remained practically unchanged in most species under aerobic conditions. In *Australorbis glabratus,* however, a rather

TABLE IV

Aerobic carbohydrate metabolism of some snail species

Species	Number of experiments	Experimental period, hours	Oxygen consumed mm³. per 1 gm.		Carbohydrates			Lactic acid in tissues microgram per 1 gm.		Oxygen required for total oxidation of consumed carbohydrates in per cent of oxygen consumed	Ratio $\frac{\text{anaerobic}}{\text{aerobic}}$ carbohydrate consumption
					Per cent in tissues		Mg. consumed per 1 gm. in 1 hour				
			Total period	1 hour	Initial	Final		Initial	Final		
Australorbis glabratus	18	16	2544 ±136	159	2.69 ±0.18	2.35 ±0.14	0.21	232 ±21	65 ±22	99	1.5
Helisoma duryi	29	24	1937 ±105	81	2.08 ±0.18	1.81 ±0.29	0.11	133 ±26	114 ± 9	104	4.1
Planorbarius corneus	18	24	2870 ±165	120	2.94 ±0.02	2.89 ±0.28	0.02	267 ±66	248 ±41	13	2.4
Lymnaea stagnalis	18	6	1230 ± 89	205	1.26 ±0.10	1.04 ±0.10	0.37	247 ±47	179 ±42	133	1.2
Lymnaea palustris	18	6	821 ±193	137	1.22 ±0.13	1.00 ±0.12	0.37	112 ±16	103 ±14	200	2.3
Pomatiopsis lapidaria	18	24	2230 ±108	93	1.47 ±0.08	1.37 ±0.08				30	4.5
Melanoides tuberculatus	18	24	1285 ± 90	54	2.73 ±0.13	2.46 ±0.16	0.11	89 ±20	98 ±33	156	1.4

sharp decline in lactic acid level occurred during the period of observation. No reason for this aberrant behavior can be given at the present time.

DISCUSSION

The fact that aquatic snails possess a certain tolerance towards the lack of oxygen has been known for some time. Alsterberg (1930) found the operculates Bulinus sp. and Vivipara sp. more resistant than the pulmonates *Lymnaea ovata, Lymnaea stagnalis,* and *Lymnaea truncatula. Planorbarius corneus* was only slightly more resistant than the lymnaeids. On the other hand, Raffy and Fischer (1933) reported Planorbarius was much more resistant than Lymnaea upon total immersion into oxygen-poor water. The present results, carried out under completely anaerobic conditions, are more in agreement with the latter than the former. In our experiments the Lymnaeidae and Physidae were considerably less resistant than the Planorbidae or the different operculates tested.

The anaerobic metabolic level of the nonresistant species, as expressed by the rates of CO_2 production and carbohydrate consumption, was, in general, somewhat higher than that of the resistant species, but a certain overlapping occurred. It is dubious, therefore, whether the metabolic level as such has a decisive bearing on the question of what factors are responsible for the differential sensitivity towards lack of oxygen. The metabolic level of snails depends, at a given temperature, on the nutritional state. Whether this last factor materially influences the length of anaerobic survival remains to be investigated.

A well defined difference between resistant and nonresistant species was found in respect to lactic acid. No accumulation whatever occurred during anaerobic periods in the tissues of the former; they excreted all the new-formed and possibly even some of the preformed lactic acid. On the contrary, the lactic acid level in the tissues of the nonresistant species increased rather sharply; these species were

capable of excreting only part of the newly formed acid. How far this accumulation of lactic acid within the tissues was responsible for the early death of the animals cannot be decided at the present time. It is hardly likely that it was the only factor involved. It must be remembered in this connection that those species which do not show such an accumulation of lactic acid ultimately die of asphyxiation.

All species consumed carbohydrate anaerobically at a somewhat faster rate than under aerobic conditions, but only in *Helisoma duryi* and *Pomatiopsis lapidaria* was the quotient $\frac{\text{anaerobic consumption}}{\text{aerobic consumption}}$ as large as that in most hitherto-studied free-living invertebrates (older literature in von Brand, 1946; newer data in Cleary, 1948). In the other species the quotient was small, in *Australorbis glabratus*, *Lymnaea stagnalis*, and *Melanoides tuberculatus* as small, indeed, as in parasitic worms. It is difficult to adduce definite reasons for this rather curious observation. A low carbohydrate consumption quotient may result under anaerobic conditions, if the snail survives by producing a relatively small amount of energy; then the anaerobic carbohydrate consumption may proceed at a relatively low rate even in comparison with the much more efficient oxidative breakdown. It seems very probable that such a reduction in energy production is involved, at least to some degree, in the present case. It is quite certain that these snails possess only a tolerance towards anaerobiosis, but are not capable of leading an anaerobic life in the true sense of the word.

However, a second factor may also be involved. One of the reasons why the above carbohydrate quotient is low in many parasitic worms is the fact that they have a metabolism characterized by aerobic fermentations, which are less efficient in energy production than completely aerobic respiration and therefore increase the aerobic carbohydrate consumption, thus lowering the quotient. Our findings concerning the quantitative relationships between oxygen and carbohydrate consumption seem to indicate that such a situation may also prevail in some snails. This point will have to be clarified by further work.

It has been previously pointed out (von Brand, Nolan, and Mann, 1948) that the aerobic metabolic levels of pulmonate and operculate snails are different, but that the oxygen consumption follows the surface law both in intraspecific and interspecific comparisons. The present study reveals that, at least, in pulmonate snails (no suitable material of operculates was available), the anaerobic carbon dioxide production follows the surface law in intraspecific comparison. This seems to be the first instance that such a relationship has been established for the anaerobic metabolism of invertebrates. The same relationship, however, definitely does not hold in all cases of interspecific comparison. The reasons for differences in anaerobic carbon dioxide excretion by various species of snails may be different. Our observations on the behavior of lactic acid revealed considerable differences between species. In Lymnaeidae and Physidae this acid accounted for a rather large percentage of the excreted carbon dioxide, in other species only for a small one. It is then quite possible that different metabolic processes lead to different rates of carbon dioxide production depending upon the proportions of inorganically and organically derived carbon dioxide. Similar considerations apply to the rates of anaerobic carbohydrate consumption, various fermentative pathways liberating different amounts of energy. Whether or not fixed relationships exist between rates of anaerobic

metabolism and size of snails belonging to different species, can probably be determined only after the actual energy production of the various processes has been elucidated.

A rather interesting result of our experiments is the observation that lactic acid, although produced by all snails, accounts in several species only for a small fraction of the anaerobically consumed carbohydrate; while in others it obviously represents a major end product. Snails had never before been studied in this respect, but similar differences are well known to occur in parasitic worms (review of the literature in Bueding, 1949), protozoa (literature in von Brand, 1950), as well as mollusks other than snails. Dugal and Fortier (1941) found no anaerobic lactic acid production in oysters, nor did Wernstedt (1944) in Dreissensia; while the production of rather large amounts of lactic acid have been reported in the case of Venus (Dugal, 1939). Recently, however, Humphrey (1949) observed some lactic acid production also in oysters.

Our studies indicate that the consumption of carbohydrate is probably of major importance in allowing snails to survive the adverse condition of lack of oxygen. It would then seem justifiable to attempt the destruction of snails that are present in anaerobic or semi-anaerobic habitats, by the application to such habitats of chemical compounds known to interfere with the glycolytic enzymes.

Summary

1. Lymnaeidae and Physidae tolerated complete lack of oxygen less well than Planorbidae or operculates belonging to different families.

2. All species consumed carbohydrate under anaerobic conditions and produced carbon dioxide and lactic acid. While in several species the lactic acid produced was sufficient to account for all or a large part of the carbon dioxide as liberated from bicarbonate, this was not the case in other species.

3. The anaerobic metabolic level as measured by carbon dioxide production and carbohydrate consumption of the resistant species was, on an average, lower than that of the nonresistant ones. The former did not accumulate lactic acid within their tissues during an anaerobic period, while the latter did so to a marked degree.

4. In most species the anaerobic carbohydrate consumption was only slightly higher than the aerobic rate. One of the reasons for this may be the probable occurrence of aerobic fermentations in these species.

5. Lactic acid was quantitatively a major end product of the anaerobic carbohydrate consumption only in *Lymnaea stagnalis* and *Lymnaea natalensis;* in all other species unidentified end products must have prevailed.

6. The anaerobic carbon dioxide production followed the surface law in intraspecific comparisons in pulmonates rather closely, but definitely not in all cases of interspecific comparisons.

Literature Cited

Alsterberg, G., 1930. Wichtige Zuege in der Biologie der Suesswassergastropoden. Lund.

Barker, S. B. and W. H. Summerson, 1941. The colorimetric determination of lactic acid in biological material. *J. Biol. Chem.*, **138**: 535–554.

von Brand, T., 1946. Anaerobiosis in invertebrates. *Biodynamica Monographs No. 4*. Biodynamica, Normandy, Missouri.

VON BRAND, T., 1950. The carbohydrate metabolism of parasites. *J. Parasitol.*, (In press).

VON BRAND, T., M. O. NOLAN, AND E. R. MANN, 1948. Observations on the respiration of *Australorbis glabratus* and some other aquatic snails. *Biol. Bull.*, **95**: 199–213.

BUEDING, E., 1949. Metabolism of parasitic helminths. *Physiol. Rev.*, **29**: 195–218.

CLEARY, M. B., 1948. The anaerobic glycogen consumption and the question of post-anaerobic glycogen resynthesis in some invertebrates. *Dissertation*, Catholic Univ. of America.

DISCHE, Z. AND H. POPPER, 1926. Ueber eine neue kolorimetrische Mikrobestimmungsmethode der Kohlehydrate in Organen und Koerpersaeften. *Biochem. Ztschr.*, **175**: 371–411.

DUGAL, L. P., 1939. Le rôle de la coquille dans le métabolisme anaérobique de *Venus mercenaria*. *Ann. de l'ACFAS*, **5**: 95.

DUGAL, L. P. AND G. FORTIER, 1941. Le métabolisme anaérobique chez les mollusques. II. Variations du calcium et de l'acide lactique chez les huitres. *Ann. de l'ACFAS*, **7**: 112.

HUMPHREY, G. F., 1949. Invertebrate glycolysis. *J. Cell. and Comp. Physiol.*, **34**: 323–325.

RAFFY, A. AND P. H. FISCHER, 1933. Utilisation de l'oxygène dissous chez les pulmonés aquatiques. *Compt. Rend. Soc. Biol.*, Paris, **112**: 782–784.

WERNSTEDT, C., 1944. Metabolism of gill epithelium of a freshwater mussel. *Nature*, **154**: 463.